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(54) Title: VACCINE CONTAINING LIVE VIRUS FOR THERAPY OF VIRAL DISEASES AND MALIGNANCIES

(57) Abstract

A process for preparing a purified virus vaccine comprises the steps of purifying a fluid containing a virus by centrifugation, ultracentrifuging to pellet the supernatant, purifying the virus by sucrose gradient ultracentrifugation, rehydration and lyophilization. Desirably, a modified starch, such as hydroxyethyl starch having a molecular weight in the range 100,000-300,000, is added as a protective colloid prior to lyophilization. The virus is selected from the group consisting of avian paramyxovirus, avian herpesvirus, avian rotavirus, avian bronchitis, avian encephalitis, avian bursitis (Gumboro) virus, Marek's disease virus, parvovirus, Newcastle disease virus, human paramyxovirus, human parvovirus, human adenovirus, and mixtures therof. A purified virus vaccine made by the foregoing method is useful for the treatment and control of mammalian disease of viral origin.

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VACCINE CONTAINING LIVE VIRUS FOR THERAPY OF VIRAL DISEASES AND MALIGNANCIES

The present invention relates to pharmaceutical products containing stabilized, live virus for the therapy of viral diseases and malignancies and to the process for the production of such products. The present invention also relates to a purified virus vaccine and the purification procedure therefor.

Hungarian Patents #197 517 and #197 846 describe the use of certain live, apathogenic viruses in the therapy of various human diseases of viral origin. Thus, patent # 197 517 provides a pharmaceutical product containing attenuated Newcastle disease virus suitable for the therapy of herpes, rabies, AIDS and malignancies. Patent # 197 846 describes a pharmaceutical product containing attenuated Gumboro virus suitable for the treatment of hepatitis, rabies, and other diseases of viral origin and malignancies. Although both Gumboro and Newcastle disease viruses cause poultry diseases, the vaccines containing these attenuated viruses are in commercial use. The above patents describe the therapeutic application of these vaccines.

Since the purity of veterinary vaccines do not meet human purity requirements, infections and complications may result as untoward side effects. Moreover, the stability of veterinary vaccines may also be poor. The present invention is intended to provide a process to obtain purified, apathogenic viruses suitable for human therapy as well as a lyophilized product which is stable for long periods without apparent loss of

effectiveness.

Recently, it has been found that other apathogenic viruses can also be used in the therapy of human diseases of viral origin. It has been proven, according to the present invention, that any attenuated virus apathogenic for humans can be used, alone or in combination, in the treatment of viral diseases. These may be veterinary, in particular, fowl viruses, or human viruses; e.g.; avian paramyxovirus, avian herpesvirus, avian rotavirus, avian bronchitis, avian encephalitis, avian bursitis (Gumboro) virus, Marek's disease virus, parvovirus, Newcastle disease virus as well as human paramyxovirus, human parvovirus and human adenovirus.

The invention relates to attenuated viruses apathogenic to humans which are effective in the treatment of diseases of viral origin and malignancies, e.g., as follows: AIDS, carcinoma of the rectum, bladder, breast, colon, cervix, esophagus, pancreas, bronchus, liver, kidney and stomach, gynecological cancers, head and neck cancers, lymphomas, malignant melanoma, myeloma, immune deficiency due to irradiation, multiple sclerosis, influenza, common cold and related diseases of viral origin, herpes genitalis and labialis, warts, collagen diseases, acute and chronic hepatitis (B and C), and symptoms following bone marrow transplantation.

The viruses suitable for the above therapeutic purposes may be obtained as usual, e.g., from fibroblast or other cell line cultures or allanto-amniotic fluid of egg embryos. The allanto-amniotic fluid can be obtained from infected hen eggs. The fluid is purified by centrifugation and the supernatant is pelleted by ultracentrifuge. The sediment is rehydrated and sedimented over sucrose gradient, ultracentrifuged again

and the pellet is rehydrated and lyophilized.

In a preferred embodiment of the invention the allantois fluid is centrifuged by approximately 5000 x g, the pellet is discarded and the supernatant is used (if necessary a filtration step can be included). The virus is pelleted from this supernatant by ultracentrifugation (the ultracentrifugation depends on the r.p.m. and time, and may vary over a wide range, usually 35 000 x g for 1 hour). The supernatant is discarded and the pellet is resuspended in a small volume of buffer solution. For appropriate homogeneity a relative longer period of mixing is required.

This homogeneous suspension is layered over a high concentration of sucrose and ultracentrifuged at 90 000 - 100 000 x g (minimal g: 60 000). The supernatant is discarded and the pellet is rehydrated and lyophilized.

Another object of the invention is stabilization of the virus preparation. Protective colloids, either alone or in combination, during lyophilization are generally used in the production of vaccines. Such colloids are, e.g., milk (3-10%), polyvinylpyrrolidone and gelatin (0.1-0.2%), and glucose, sucrose or dextran (1-10%). However, for human use, these colloids are either unsatisfactory or may cause side effects.

We have found that modified starch, either alone or in combination, can preferably be used as the protective colloid, such as hydroxyethyl starch (molecular weight: 100 000 - 300 000). Hydroxyethyl-starch of an average molecular weight of 200 000 is used as plasma expander, but such compounds have not been used as protective colloids for vaccine production.

The new stabilized product according to this invention contains, together with other compounds, an effective amount of modified starch as

the protective colloid.

The invention will be detailed in the following examples. Newcastle disease and Gumboro virus can be purchased from Phylaxia of Budapest, Hungary as PHYLAVAC and GUMBOPHYL, respectively.

Example #1. Purification of Newcastle disease virus from allantois fluid

Three liters of allanto-amniotic fluid containing the virus were centrifuged at 5000 x g for 1 hour. The supernatant was filtered through multiple layers of gauze. The virus was pelleted from the supernatant by ultracentrifugation (SCP 85 H2 ultracentrifuge, RP 19 rotor, 18 500 rpm (35 000 x g, 4°C, 1h)). After discarding the supernatant, the pellet was resuspended in 30 ml NTE buffer (0.15 M NaCl, 0.001 M EDTA, 0.05 M TRIS; pH 7.4). The suspension was gently mixed for 24 hours in an ice bath.

The suspension was further purified by sucrose gradient ultracentrifugation. Thirty ml of 30% (w/w) (=33% w/v) sucrose in NTE buffer was placed into centrifuge tubes and 5 ml of suspension was layered onto the sucrose. The tubes were ultracentrifuged in an SRP rotor at 95 000 x g (27 500 rpm) for 80 min.

After discarding the supernatants, the pellets were resuspended in NTE buffer (0.5 ml/tube). The collected supernatants were gently mixed for 24 hours in an ice bath.

The concentration of virus during the purification procedure was checked by neuraminidase activity, hemagglutination and ELISA. The infectivity of the virus was measured by the inoculation of preincubated eggs. The protein concentration was measured by the method of Spector. The purity of the product was checked by SDS gel electrophoresis; except

for HN, NP and M proteins no other bands (contaminants) should be seen.

The above method displayed the following features:

	Volume	ELISA (HI)	yield %
Original material	3 l	154	100
Supernatant	3 l	1	0.06
Resuspended pellet	42 ml	9531	87
Supernatant over sucrose	310 ml	467	31
Purified virus	11 ml	20803	50

Example #2. Purification of Gumboro virus from Vero cell culture

2300 ml supernatant of Vero cell culture was centrifuged for 30 min at 5000 x g at 4°C. Virus was pelleted from the supernatant by ultracentrifugation (SCP 85 H2 ultracentrifuge, RP 19 rotor, 18 500 rpm (35 000 x g, 4°C, 1h)). After discarding the supernatant, the pellets were resuspended in 23 ml NTE buffer (1% of the original volume). The suspension was gently mixed for 24 hours in an ice bath.

The suspension was further purified by sucrose gradient ultracentrifugation. Thirty ml of 30% (w/w) (=33% w/v) sucrose in NTE buffer was placed into centrifuge tubes and 5 ml of suspension was layered onto the sucrose. The tubes were ultracentrifuged in SRP 28SA rotor at 95 000 x g (27 500 rpm) for 80 min.

After discarding the supernatants, the pellets were resuspended in NTE buffer (1 ml/tube), then washed with 1 ml buffer. The collected

supernatants were gently mixed for 24 hours in an ice bath.

The concentration of virus during the purification procedure was checked by ELISA. The infectivity of the virus was measured by its cytopathogenic effect. The protein concentration was measured by the method of Spector.

The above described method displays the following features:

	volume	ELISA (HI)	yield %
Original material	2300 ml	171	100
After centrifugation	2300 ml	133	78
Supernatant	2300 ml	48	28
Resuspended pellet	28 ml	3621	26
Supernatant over sucrose	180 ml	212	10
Purified virus	13 ml	5271	17

Example #3. Stabilized virus for human therapeutic use

2-2% (v/v) glucose, sucrose and hydroxyethyl-starch (mw: 200 000) (ISOHES, HES 200/0.5) were added to the virus suspension obtained from example #1, then lyophilized. After reconstitution, even after prolonged storage, the original ELISA titre was obtained.

CLAIMS

1. A process for preparing a purified virus vaccine, comprising the following steps:

a. purifying a fluid containing a virus by centrifugation;

b. performing ultracentrifugation to pellet a supernatant; and

c. purifying the virus by sucrose gradient ultracentrifugation, rehydration, and lyophilization.

2. The process of claim 1 wherein a modified starch is added as a protective colloid just prior to lyophilization.

3. The process of claim 2 wherein said modified starch is a hydroxyethyl starch of 100,000-300,000 molecular weight.

4. The process of claim 1 wherein said fluid containing the virus is an allanto-amniotic fluid.

5. The process of claim 1 wherein said virus is selected from the group consisting of avian paramyxovirus, avian herpesvirus, avian rotavirus, avian bronchitis, avian encephalitis, avian bursitis (Gumboro) virus, Marek's disease virus, parvovirus, Newcastle disease virus, human paramyxovirus, human parvovirus, human adenovirus, and mixtures thereof.

6. A purified virus vaccine prepared by a process comprising the steps of:

a. purifying a fluid containing a virus by centrifugation;

b. performing ultracentrifugation to pellet a supernatant; and

c. purifying the virus by sucrose gradient ultracentrifugation, rehydration, and lyophilization.

7. A purified virus vaccine as in claim 6 containing a virus apathogenic for humans.

8. A purified virus vaccine as in claim 7 wherein said virus is an avian bursitis (Gumboro) virus.

9. A purified virus vaccine as in claim 7 wherein said virus is a Newcastle disease virus.

10. A purified virus vaccine as in claim 6 wherein said virus is selected from the group consisting of avian paramyxovirus, avian herpesvirus, avian rotavirus, avian bronchitis, avian encephalitis, Marek's disease virus, parvovirus, human paramyxovirus, human parvovirus, human adenovirus, and mixtures thereof.

11. A method for the treatment and control of mammalian disease of viral origin characterized by the administration to a host in need of such treatment of an effective amount of a vaccine according to claim 6.

12. A purified virus vaccine characterized by a stable virus preparation utilizing a modified starch either alone or in combination as a protective colloid.

13. A purified virus vaccine as in claim 12 containing a virus apathogenic for humans.

14. A purified virus vaccine as in claim 13 wherein said virus is an avian bursitis (Gumboro) virus.

15. A purified virus vaccine as in claim 13 wherein said virus is a Newcastle disease virus.

16. A purified virus vaccine as in claim 12 wherein said virus is selected from the group consisting of avian paramyxovirus, avian herpesvirus, avian rotavirus, avian bronchitis, avian encephalitis, Marek's disease virus, parvovirus, human paramyxovirus, human parvovirus, human adenovirus, and mixtures thereof.

17. A purified virus vaccine as in claim 12 wherein said modified starch is hydroxyethyl starch having a 100,000-300,000 molecular weight.

18. A method for the treatment and control of mammalian disease of viral origin characterized by the administration to a host in need of such treatment of an effective amount of a vaccine according to claim 12.

19. A purified virus vaccine for use in the treatment of mammalian disease of viral origin, which comprises as an active ingredient a therapeutically effective amount of an attenuated apathogenic virus in association with a pharmaceutically acceptable carrier.

20. The product of the process of claim 3.

INTERNATIONAL SEARCH REPORT

International application No.
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A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 39/12, 39/15, 39/155, 39/17, 39/215, 39/255; C12N 07/02
US CL :424/89; 435/239
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/89, 93T; 435/239

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CA, Medline, Pascal, Biosis, Scisearch, Life Sciences Collection

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	See Attached Sheet.	

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	H. Fraenkel-Conrat et al, "Virology" published 1982 by Prentice-Hall, Inc. (N.J.), pages 17-20, see entire document.	1,5,6-10,16,20 4
X,P	US, A, 5,124,148 (Csatary et al) 23 June 1992, see column 2, lines 53-68 and column 3, lines 1-35.	11,18,19
Y	US, A, 4,824,668 (Melchior et al) 25 April 1989, see column 1, lines 65-68 and column 2, lines 1-23, and column 4, lines 13-21.	2,3,12-15,17
Y	US, A, 4,457,916 (Hayashi et al) 03 July 1984, see column 3, lines 6-14.	2,3,12-15,17
Y	US, A, 4,158,054 (Furminger et al) 12 June 1979, see column 2, lines 25-65.	4
Y	US, A, 4,235,876 (Gits et al) 25 November 1980, see column 2, lines 42-46.	15